PATENT
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Applicants	:	Rosenblatt et al.
Serial No.	:	09/016,743
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For	:	CHIMERIC ANTIBODY FUSION PROTEINS FOR THE RECRUITMENT AND STIMULATION OF AN ANTITUMOR IMMUNE RESPONSE

Examiner: Larry R. Helms, Ph.D.

Art Unit: 1642

DECLARATION OF SEUNG-UON SHIN UNDER 37 C.F.R. § 1.132

U.S. Patent and Trademark Office
P.O. Box 2327
Arlington, VA 22202

Dear Sir:

I, Seung-Uon Shin, pursuant to 37 C.F.R. § 1.132, declare:

1. I hold a Ph.D. degree in Cell Biology from Albert Einstein College of Medicine, New York, New York.

2. I am a Research Associate Professor at the University of Miami School of Medicine.

3. I am a named inventor of the above-identified patent application.

4. I am submitting this declaration to demonstrate that scientists skilled in the field of antibody-based cancer therapeutics would not regard information relating to immunoconjugates of single chain Fv analogs as relevant to immunoconjugates made from whole antibodies. As explained below, there are significant differences with regard to the avidity, half life, and chemokine carriage which would cause scientists skilled in the field of antibody cancer therapeutics to avoid adapting single chain Fv analog technology to whole antibody cancer therapeutics.

Avidity

5. Whole antibodies have two binding sites, while single chain Fv analogs have one binding site. Although they have exactly the same affinities, whole antibodies show higher binding ability to antigen than single chain Fv analogs, because of the avidity of the former. This characteristic of whole antibodies will provide stronger binding to antigen than single chain Fv analogs. As a result, diffusion of whole antibody therapeutics into tumors is prevented and, by remaining on the surface of solid tumors, such therapeutics will have the tendency to achieve better recruitment of immune effector cells from the blood. Since it is very hard to obtain any antibody with an affinity of $>1 \times 10^{-10}$ M, the diminished affinity of single chain Fv analogs will tend to impair their ability to bind to tumors avidly *in vivo*.

6. The difference in avidity between whole antibodies and single chain Fv analogs is demonstrated by Adams et al., "High Affinity Restricts the Localization and Tumor Penetration of Single-Chain FV Antibody Molecules," Cancer Res, 61(12):4750-5 (2001) ("Adams")(attached as Appendix A). In this paper, a series of affinity mutants of the C6.5 single chain Fv analogs demonstrated that high affinity limited tumor localization and intratumoral diffusion of small antibody-based molecules. In biodistribution studies, quantitative tumor retention of the radiolabeled single chain Fv analogs molecules plateaued at affinities $>1 \times 10^{-9}$ M. Adams observed that a low affinity (3.2×10^{-7} M) anti-HER-2/neu single chain Fv analogs exhibited broad diffusion from the vasculature into the tumor, whereas the highest affinity (1.5×10^{-11} M) single chain Fv analogs generally failed to traverse more than 2-3 cell diameters. The moderately high affinity (1×10^{-9} M) single chain Fv analogs revealed an intermediate staining pattern. These studies indicate that the affinity for HER-2/neu dictates the degree of single chain Fv analogs' penetration from blood vessels into tumors. However, antibodies of low affinity may diffuse too rapidly and fail to provide for the formation of a chemokine gradient at the site of tumors.

Half-life

7. The half-life of whole antibodies is generally much longer than that of single chain Fv analogs. This prolonged half-life of antibodies increases bioavailability to tumors. Since single chain Fv analogs possess a short half-life, they must be frequently administered at a high dosage to achieve a desired anti-tumor efficacy.

8. The difference in half-life between whole antibodies and single chain Fv analogs is demonstrated by Covell et al., "Pharmacokinetics of Monoclonal Immunoglobulin G1, F(ab')2, and Fab' in Mice," Cancer Res, 46(8):3969-78 (1986)("Covell") (attached as Appendix B) and Goel et al., "^{99}mTc-Labeled Divalent and Tetravalent CC49 Single-Chain Fv's: Novel Imaging Agents for Rapid In Vivo Localization of Human Colon Carcinoma," J Nucl Med, 42(10):1519-27 (2001)("Goel") (attached as Appendix C).

9. As demonstrated by Goel, whole-body clearance studies confirmed the rapid elimination of single chain Fv analogs with half-lives of 184 +/- 19 min for single chain $(Fv)_2$ analogs and 265 +/- 39 min for [single chain $(Fv)_2$ analogs]$_2$. Thus, whole antibody fusions should have a longer half life than single chain Fv analogs.

10. As shown by Covell, whole IgG remains in the body for 8.3 days, but the Fab' fragment is cleared from the body 35 times faster than whole antibody. The F(ab')2 fragment has pharmacokinetic characteristics that fall between those of whole IgG and Fab'. These results provide pharmacokinetic criteria for selecting whole IgG, F(ab')2, or Fab' for various *in vivo* applications. Single chain Fv analogs are more likely to behave like Fab', while whole antibodies should have an increased half-life.

Carriage of Two Chemokine Molecules

11. Whole antibodies can carry two molecules of chemokines, but single chain Fv analogs carry only a single chemokine. Since chemokine receptors can form dimers, whole antibody fusion proteins carrying two chemokines would be much more effective cancer therapeutics than single chain Fv analog fusions. In addition, two chemokine molecules provide stronger binding to their receptors than single chain Fv analogs, which would be dimerized after chemokine binding to transmit intracellular signals.

12. G-protein coupled receptors constitute a large family of homologous transmembrane proteins which are activated by a variety of different ligands such as chemokine, neurokinin, opioid, somatostatin, thyrotrophinm, and the whole biogenic amine family. G-protein coupled receptor can dimerize with the dimer being the functionally active form of the receptor.

13. Thus, an antibody carrying two chemokine molecules would tend to be more efficient at signaling through facilitation of receptor dimerization and/or crosslinking.

This is demonstrated by Gouldson et al., "Lipid-Facing Correlated Mutations and Dimerization in G-Protein Coupled Receptors," Protein Eng, 14(10):759-767 (2001)("Gouldson")(attached at Appendix D) and Vila-Coro et al., "The Chemokine SDF-1alpha Triggers CXCR4 Receptor Dimerization and Activates the JAK/STAT Pathway," FASEB J, 13(13):1699-710 (1999)("Vila-Coro")(attached at Appendix E).

14. As shown in Gouldson, the formation of heterodimers or homodimers (which may be domain swapped) or oligomers are required for activation or internalization of receptors. The results are discussed in the light of the subtype-specific heterodimerization observed for the chemokine, opioid, and somatostatin receptors. The CCR2 subtype will heterodimerize with CCR5 but not CXCR4.

15. Vila-Coro demonstrates that chemokine receptors are members of the seven-transmembrane domain G-protein-coupled receptors, which have also been divided in two main subfamilies (CXCR and CCR), depending on their chemokine specificity. As occurs with the cytokine receptors in response to cytokines, the CXCR4 undergoes receptor dimerization after SDF-1 binding and is a critical step in triggering biological responses. Thus, presentation of 2 chemokine molecules fused to a whole antibody should be more efficient at eliciting receptor dimerization and triggering chemotactic response.

16. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 7-18-2002

Seung-Uon Shin